· PRELIMINARY PROTOCOL for Odyssey In-Gel Western Detection – June 2001

1. Following electrophoresis, separate the two plates. If desired, the stacking gel may be cut away from the top of the gel using a scalpel or razor blade (it will exhibit high background when gel is scanned).
2. Incubate the gel in 50% isopropanol (prepared with ultrapure water) for 15 minutes. Shake gently, and use enough solution that the gel is completely covered and can move freely.
3. Remove isopropanol and wash the gel in ultrapure water for 15 minutes with gentle shaking. Use enough water that the gel is completely submerged and can move freely. The gel may curl and/or float to the surface. Gently flatten the gel and be sure that it is completely covered; it is important to remove residual alcohol.
4. If desired, you may stop here and store the gel in water overnight at 4^{o}C. No blocking step is required.
5. Dilute primary antibody to desired concentration in Odyssey Blocking Buffer or PBST. Since in-gel detection is not as sensitive as a standard Western blot, you may wish to use more primary antibody than usual. Make sure that the gel is completely covered by antibody solution. Incubate gel in primary antibody for 1 hour with gentle shaking.
6. Primary antibody incubation can be extended to several hours, or carried out overnight at 4^{o}C. Extended incubation will generally increase signal.
7. Wash the gel 3 x 10 minutes in PBS + 0.05 - 0.1% Tween with gentle shaking.
8. Dilute Odyssey secondary antibody at 1:1000 - 1:5000 in Odyssey Blocking Buffer or PBST. Incubate gel in secondary antibody for 1 hour with gentle shaking, and protect from light. Again, use enough antibody solution to completely cover gel.
9. Wash the gel 3 x 10 minutes in PBS + 0.05 - 0.1% Tween with gentle shaking.
10. Wash the gel for 5 minutes in PBS.
11. Lay the wet gel on the scanner glass of the Odyssey instrument. For scanning, set the focus offset to _ the thickness of the gel. If the stacking gel was not removed from the top of the gel, it will show dark background. Crop the image to remove this background.
12. The gel may be stored at 4^{o}C for several days, if protected from light.

Notes on the protocol:

This is a PRELIMINARY PROTOCOL and may require optimization in your hands.

Optimization of primary and secondary antibody dilutions, as well as amounts of Tween, may be needed to achieve maximum signal and minimum background.

Try different buffers for dilution of your antibodies, including PBST alone, Odyssey Blocker, milk, BSA, Pierce SuperBlock, etc. (Although you should not use BSA for blocking of ***membranes*** for Odyssey, it can be used for in-gel Westerns.)

Expect the detection sensitivity to be somewhat lower than a standard Western blot. Transfer to a membrane concentrates the target protein; while still in the gel, protein is dispersed through the thickness of the gel.

In-gel detection may not be quantitative.

Gel thickness may affect the ability of antibody molecules to penetrate the gel. We use 1.0 mm gels.

The performance of different pre-cast gels may vary widely. We have the best success with Novex Tris-glycine gels.

In-gel Westerns may be stripped and reprobed. Use the same buffer you would use for stripping of an Odyssey Western, but carry out stripping for 30-60 minutes.